JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1998, p. 783–787 Vol. 36, No. 3
0095-1137/98/$04.00+0

Biochemical and Pathogenic Properties of *Shewanella alga* and *Shewanella putrefaciens*

SHIDEH KHASHE AND J. MICHAEL JANDA*

Microbial Diseases Laboratory, Division of Communicable Disease Control, California Department of Health Services, Berkeley, California 94704-1011

Received 10 October 1997/Returned for modification 24 November 1997/Accepted 15 December 1997

We characterized 49 strains of *Shewanella* spp. from clinical ($n = 31$) and nonhuman ($n = 18$) sources. Most *Shewanella alga* organisms (Gilardi biovar 2; Centers for Disease Control and Prevention [CDC] biotype 2) originated from clinical material (92%), failed to produce acid from carbohydrates other than D-ribose, and were biochemically and enzymatically fairly homogeneous. In contrast, *Shewanella putrefaciens* organisms (Gilardi biovars 1 and 3; CDC biotype 1) were more often associated with nonhuman sources (70%), were able to utilize a number of sugars (sucrose, L-arabinose, and maltose), and were found to exhibit wider variations in biochemical characteristics; three biotypes within *S. putrefaciens* were detected. Notable differences between the two species in enzymatic activity, determined with the API-ZYM system (bioMérieux, Hazelwood, Mo.), and cellular fatty acid profiles, determined by the MIDI system (Microbial ID Inc., Newark, Del.), were also detected. Pathogenicity studies of mice indicate that *S. alga* appears to be the more virulent species, possibly due to the production of a hemolytic substance.

The taxon *Shewanella putrefaciens* ("*Pseudomonas putrefaciens*") comprises a group of gram-negative oxidative and nonoxidative bacilli whose chief phenotypic attribute is the production of hydrogen sulfide gas (H_2S) on TSI slants (22). Although *S. putrefaciens* has been implicated occasionally as a human pathogen, it is most frequently recovered from nonhuman sources, including aquatic reservoirs (marine, freshwater, and sewage), natural energy reserves (oil and gas), soil, and fish, poultry, dairy, and beef products (14, 18, 20, 21). Most human isolates of *S. putrefaciens* occur as part of a mixed bacterial flora, clouding their clinical significance. However, a number of monomicrobic illnesses due to *S. putrefaciens* have been documented and include bacteremia, soft tissue infections, and otitis media (1, 2, 4, 13).

For over two decades, it has been known that *S. putrefaciens* is a genetically heterogeneous species. These conclusions are based upon the wide variation in G+C content (44 to 54 mol%), results of DNA-DNA reassociation studies, and numerical taxonomy investigations (14, 17, 18, 20, 21). As late as the 1980s, Gilardi (6) recognized three distinct biovars within *S. putrefaciens*, while the Centers for Disease Control and Prevention (CDC) (22) recognized two biotypes based upon carbohydrate oxidation patterns and growth on salmonella-shigella (SS) medium and nutrient agar containing high salt (~6%) concentrations. In 1990, Simidu et al. (19) proposed the name *Shewanella alga* for a tetrodotoxin-producing isolate recovered from red algae. Subsequent to this publication, Nozue and colleagues (15) found that strains of *S. putrefaciens* with high G+C contents (52 to 54 mol%) were genetically related to *S. alga* and not to the type strain of *S. putrefaciens* (ATCC 8071). Furthermore, the latter study found that all *S. alga* strains produced a hemolytic reaction on sheep blood agar while *S. putrefaciens* isolates lacked this activity.

Of 40 clinical isolates of *S. putrefaciens* reidentified by Nozue and others (15), 33 (83%) were found to be *S. alga* based upon DNA homology values and phenotypic criteria. Domínguez et al. (5) later reported the first two Danish cases of *S. alga* bacteremia, which had originally been mistakenly attributed to *S. putrefaciens*. A follow-up 1997 systematic investigation of 76 *Shewanella* strains found that 16 of 19 human isolates (84%) resided within the *S. alga* group, based upon various taxonomic criteria including 16S rRNA sequencing, ribotyping, and whole-cell protein profiles (21). These studies suggest that *S. alga* may be the predominant human pathogen within the genus.

Presently, it is unclear how easy it is to distinguish *S. putrefaciens* from *S. alga* in the clinical laboratory and whether or not differences in host tropism do occur and correlate with pathogenic characteristics and overt virulence. In light of these questions, we have analyzed the biochemical and enzymatic capabilities of 49 *Shewanella* strains from diverse sources (human and nonhuman) to address these issues and have performed pathogenicity studies on representative strains from each group. These studies serve as the basis of this report.

MATERIALS AND METHODS

Bacterial strains. Forty-nine strains of *Shewanella* spp. (human [$n = 31$] and nonhuman [$n = 18$]) were investigated in this study. Five of these strains were cultures submitted to the Microbial Diseases Laboratory for identification. Two reference strains (ATCC 8071 and ATCC 49138) were obtained from the American Type Culture Collection (Rockville, Md.). The remaining 42 reference or wild-type strains were kindly provided by the following persons: A. Carnahan (Baltimore, Md.), A. Chipman (West Sacramento, Calif.), J. Graf (Bern, Switzerland), C. Kaysner (Bothell, Wash.), V. Knight (New Brunswick, N.J.), D. Lies (Milwaukee, Wis.), V. Pünter (Zurich, Switzerland), and T. Robin (New York, N.Y.). Most of these strains were received as *S. putrefaciens*. Working cultures were maintained in motility deeps at room temperature; typically, these isolates produced an orangish discoloration at the surfaces of these deeps upon prolonged storage. All assays were performed at 35°C, with the exception of eight strains recovered from Lake Michigan, which grew poorly or failed to grow at elevated temperatures; these strains were tested at 25°C.

Identification. Isolates that were motile, had oxidative metabolisms, were oxidase and catalase positive, ornithine decarboxylase positive, and DNase positive, and produced H_2S on triple sugar iron slants within 72 h of incubation were identified as belonging to the phenospecies *S. putrefaciens*. Several *Shewanella* strains failed to grow on DNase test agar (Difco Laboratories, Detroit, Mich.), and six isolates were nonmotile. All other reactions were uniformly positive for the 49 *Shewanella* strains studied. The biovar and biotype of each isolate were

* Corresponding author. Mailing address: Microbial Diseases Laboratory, 2151 Berkeley Way, Berkeley, CA 94704-1011. Phone: (510) 540-2242. Fax: (510) 540-2374. E-mail: jjanda@hw1.cahwnet.gov.

TABLE 1. Biovar, biotype, and species designations of *Shewanella* isolates according to various schemata

Source and schema	No. (%) of *Shewanella* isolates from:	
	Humans (n = 31)	Nonhuman sources (n = 18)
Gilardi (6)		
Biovar 1	5 (16)	12 (67)
Biovar 2	23 (74)	2 (11)
Biovar 3	3 (10)	4 (22)
Weyant et al. (22)		
Biotype 1	5 (16)	12 (67)
Biotype 2	23 (74)	2 (11)
Ungrouped	3 (10)	4 (22)
Nozue et al. (15)		
S. alga	24 (77)	2 (11)
S. putrefaciens	7 (23)	16 (89)

determined according to Gilardi (6) and Weyant et al. (22), respectively, based on acid production from sucrose and maltose, growth on SS agar, and growth in the presence of 6.5% NaCl. Species designations were determined by the criteria of Nozue et al. (15) by using the following tests: hemolysis on sheep blood agar, growth at 42°C, growth on nutrient agar containing 6.5% NaCl, growth on SS agar, and acid production from maltose and L-arabinose. Five strains each of *S. alga* and *S. putrefaciens* of clinical origin were also tested on the API 20E (bioMérieux, Hazelwood, Mo.), API NFT (bioMérieux), RapID NF Plus (Innovative Diagnostic Systems, Norcross, Ga.), and Vitek (bioMérieux) systems. All tests were performed according to the manufacturers' instructions.

Routine biochemical test results were read daily for 72 h; oxidation of various carbohydrates was assessed in Difco O/F medium after 7 days of incubation. Gluconate oxidation was determined only at 48 h, as previously described (10). Enzymatic plate assay results were read daily for 7 days; appropriate positive and negative control strains were included for each assay. All of these test procedures have been described previously (8, 11, 16). Some additional enzymatic activities (2 h), gelatinase activity (3 days), and tartrate utilization (5 days) were determined with Wee-Tab tablets or gelatin strips (Key Scientific Products, Roundrock, Tex.).

Phenotypic characterization. Seventeen *Shewanella* strains (*S. alga* [n = 8] and *S. putrefaciens* [n = 9]) were evaluated further for production of a number of enzymatic activities with the API-ZYM test (bioMérieux), according to the manufacturer's instructions. Relative activity was defined as the summation of numerical values for each isolate divided by the total number of strains tested for each species (8). Similarly, the cellular fatty acid profiles for 14 *Shewanella* strains (*S. alga* [n = 7] and *S. putrefaciens* [n = 7]) were determined by the MIDI microbial identification system (Microbial ID Inc., Newark, Del.).

Virulence-associated factors and resistance markers. Ten *Shewanella* strains (*S. alga* [n = 5] and *S. putrefaciens* [n = 5]) were evaluated for potential virulence-associated characteristics, including resistance markers to select antimicrobial agents. MICs of penicillin, ampicillin, and tetracycline were determined for each strain by using the AB Biodisk E test (Remel, Lenexa, Kans.), as previously described (11). Hemolytic activity was tested by plate and broth assays and an agar overlay method; a strain of *Edwardsiella tarda* expressing a cell-associated hemolysin served as a positive control (9). The adhesive, invasive, and cytotoxic capabilities of each *Shewanella* isolate were evaluated with HEp-2 cell monolayers prepared in chamber slides (Lab Tek; Miles Laboratory, Naperville, Ill.), as previously described (7). The relative pathogenicity of each *S. alga* and *S. putrefaciens* strain was determined by 50% lethal dose (LD_{50}) assays with female Swiss Webster mice (12).

RESULTS

Biotyping of *Shewanella* isolates according to two separate schemes provided similar results (Table 1). Most clinical isolates (74%) belonged to Gilardi biovar 2 (CDC biotype 2), being sucrose and maltose negative while growing on SS agar and on media containing high NaCl concentrations. The only major difference noted between the Gilardi classification scheme (6) and that of Weyant et al. (22) was that biovar 3 isolates (sucrose, maltose, SS, and NaCl negative) were ungrouped by the CDC biotyping system. In contrast to the human isolates, biovar 1 (CDC biotype 1) predominated (67%) among nonhuman strains. These strains typically produced acid from maltose and/or sucrose and failed to grow on high salt-containing or SS agar. Based upon the recent taxonomic proposals of Nozue and others (15), biovar 2 (CDC biotype 2) strains would be identified as *S. alga* while all biovar 1 (CDC biotype 1) and 6 of 7 biovar 3 strains would be designated *S. putrefaciens*; the remaining biovar 3 strain was subsequently identified as *S. alga*. Clinically, *S. alga* was found to predominate (77%), while the majority of nonhuman isolates (89%) were confirmed to be *S. putrefaciens* (Table 1).

All 10 *Shewanella* isolates, when tested on the API 20E, API NFT, RapID NF Plus, and Vitek systems, were identified as *S. putrefaciens*, with one exception. Four of five *S. putrefaciens* isolates produced unacceptable profile numbers on the API 20E system (no. 0602026 and 0602006); the fifth strain generated a rare biotype number for *S. putrefaciens*. All *S. alga* strains yielded excellent identification as *S. putrefaciens* on the API 20E. The API NFT system identified all 10 *Shewanella* isolates as *S. putrefaciens* with good to excellent identifications, with one exception, also an *S. putrefaciens* strain (low discriminatory value, 48 h). RapID NF Plus identified all 10 *Shewanella* isolates as *S. putrefaciens*, with 99.9% accuracy. Similarly, all strains were identified by Vitek (97 to 99% accuracy) as *S. putrefaciens*, although three *S. putrefaciens* strains required 5 to 9 h of incubation before final identification, in contrast to the 4-h results for the other 7 strains. Carbohydrate reactions (arabinose and maltose) on the API 20E, API NFT, and Vitek systems, however, do permit most strains to be correctly assigned to the relevant taxa (*S. putrefaciens* and *S. alga*) if read manually after final identifications as *S. putrefaciens.*

Comparison of the biochemical and enzymatic properties of *Shewanella* species revealed a number of differences (Table 2). Hemolysis on sheep blood agar, as reported by Nozue et al. (15), was detected in all *S. alga* strains but only in a couple of *S. putrefaciens* isolates. Most *S. alga* strains exhibited this phenotype only after prolonged incubation (48 to 72 h), and the area of hemolysis was often irregular and difficult to detect. Other activities previously found to aid in the separation of *S. alga* and *S. putrefaciens*, such as growth at 42°C, growth on media containing high salt (6.5%) concentrations, and acid production from L-arabinose, sucrose, and maltose, were confirmed. We found a substantially larger number of *S. putrefaciens* strains that grew on SS agar than previously reported; most of these originated from nonhuman sources. With the exception of ribose, production of acid from carbohydrate oxidation was uniquely associated with *S. putrefaciens*. Sugar patterns, however, varied considerably among these isolates, with some being arabinose, maltose, and sucrose positive while others were positive for maltose only or were asaccharolytic (biovar 3 strains). Several new enzymatic activities were detected among select *Shewanella* isolates that to our knowledge have not been previously reported. These included tyrosinase, alkylsulfatase, chitinase, and elastase activities (Table 2); most of these enzymes were found in nonhuman isolates of *S. putrefaciens*. Most *S. alga* and *S. putrefaciens* strains produced a siderophore, as determined by Chrome Azurol S assays. This activity was weak, and five isolates (three *S. alga* and two *S. putrefaciens* isolates) failed to grow on this medium.

Select *Shewanella* isolates that grew well at 35°C were further characterized for enzymatic activity by using API-ZYM (Table 3) and for cellular fatty acid profiles with the MIDI system. Of the nine substrates attacked by one or both *Shewanella* species with API-ZYM, higher overall activities for seven of these enzymes were associated with *S. alga*. The single activity found to be stronger in *S. putrefaciens* was valine arylamidase, although this activity was extremely weak even in

TABLE 2. Biochemical and enzymatic properties of *S. alga* and *S. putrefaciens*

Test or characteristic	Cumulative % positive					
	S. alga (n = 26)			*S. putrefaciens* (n = 23)		
	24 h	48 h	Delayed (3–7 days)	24 h	48 h	Delayed (3–7 days)
Growth						
42°C[a]	96	96	96	4	4	4
SS agar[a]	100			43	52	52
6.5% NaCl[a]	88	96	100	0	0	0
Hydrolysis of:						
Esculin	0	0	0	0	0	0
Urea	8	8	8	0	0	0
Pigmentation on L-tryptophan agar	0	0	58	0	0	49
Utilization of:						
Acetate	96	100		96	100	
Citrate	4	4	4	17	17	17
D,L-Lactate	77	92	92	22	39	87
Urocanic acid	0	26	100	0	4	52
Gluconate	0	0	0	0	0	0
H_2S (cysteine)	96	96	96	83	91	91
Acid produced from:						
Arabinose[a]	0	0	0	39	49	57
Cellobiose	0	0	0	0	0	0
Galactose	0	0	0	0	0	0
Glucose	0	0	0	0	0	39
Lactose	0	0	0	0	0	0
Levulose	0	0	0	0	9	9
Maltose[a]	0	0	0	4	70	74
Mannose	0	0	0	0	9	9
Sucrose[a]	0	0	0	26	35	35
Trehalose	0	0	0	0	0	0
Ribose	0	0	35	0	0	0
Xylose	0	0	0	0	0	0
Enzymes (plate)						
Amylase	ND[b]	0	ND	ND	0	ND
Chitinase	0	0	0	26	26	39
Chondroitinase	0	0	0	0	0	0
Egg yolk (clearing)	42	87	96	0	9	43
Elastase	0	0	0	0	0	13
Hemolysis[a]	0	46	100	0	0	13
Hyaluronidase	0	0	0	0	0	0
Lipase (Tween 20)	0	96	96	0	60	96
Lipase (Tween 85)	0	0	0	0	0	0
Mucinase	0	0	0	0	0	0
Pectinase	0	0	0	0	0	0
Phosphatase	100			100		
Protease	58	92	100	9	78	98
RNase	100			59	95	100
Siderophore	0	4	87	0	0	76
Stapholysin	0	0	0	0	0	0
Sulfatase						
Alkyl-	4	4	8	0	33	73
Aryl-	0	0	0	0	0	0
Degradation of:						
L-Tyrosine	0	0	27	0	0	0
Testosterone	0	0	0	0	0	0
Xanthine	0	0	0	0	0	0
Keratin	0	0	0	0	0	0
Enzymes (key)						
α-Glucosidase	0			39		
β-Glucosidase	0			0		
α-Fucosidase	0			0		
Aminopeptidase	100			100		
Tartrate	0	0	0	0	0	0
Gelatinase	84	92	96	65	83	91

[a] Test previously found useful in the identification of *S. alga* and *S. putrefaciens*.
[b] ND, not done.

TABLE 3. Enzymatic properties of *Shewanella* species as determined by the API-ZYM test

Enzyme	*S. alga* (n = 8)		*S. putrefaciens* (n = 9)	
	% Positive	Avg activity	% Positive	Avg activity
Alkaline phosphatase	100	5.0	100	5.0
Esterase lipase (C8)	100	3.1	100	2.8
Leucine arylamidase	100	4.6	100	2.7
Valine arylamidase	13	0.1	67	1.0
Trypsin	100	5.0	78	1.8
Chymotrypsin	100	5.0	89	4.4
Acid phosphatase	100	4.4	100	2.2
Naphthol-AS-BI-phosphohydrolase	100	4.5	100	2.7
N-Acetyl-β-glucosidase	100	4.0	56	2.3

these strains. Both species produced uniformly strong alkaline phosphatase activity. An additional observation was that all *S. alga* strains consistently produced eight of these nine enzymes, the only exception being valine arylamidase. In contrast, *S. putrefaciens* was more heterogeneous, with four of the nine enzymes detected being not universally present in all isolates. Analysis of 14 *Shewanella* strains indicated that the predominant fatty acids were i-15:0, 17:1ω8c, and 16:0; some strains produced large amounts of 16:1ω7c (9 to 18%), while others produced negligible quantities. While most fatty acid peaks were fairly consistent among *S. alga* and *S. putrefaciens* strains tested, several differences were noted (Table 4). Higher mean values of pentadecanoic acid and *cis*-9-heptadecenoic acid (17:1ω8c) were noted for *S. alga*, while the converse held true for *S. putrefaciens* regarding hexadecanoic and dodecanoic acids. For hexadecanoic acid, no overlap in the total fatty acid range between *S. alga* and *S. putrefaciens* was observed; for pentadecanoic acid and 17:1ω8c, only one *S. putrefaciens* or *S. alga* isolate produced a value that fell within the other's range. While no single peak was diagnostic, the use of all four peaks together clearly separated the 14 *Shewanella* strains into two groups along species lines.

It was found that the 23 strains of *S. putrefaciens* could be broken down into three separate groups based upon several phenotypic characteristics (Table 5). Group 1, which consisted of eight strains, including ATCC 8073, produced acid from maltose, sucrose, and arabinose and utilized urocanic acid. Group 1 strains were equally divided among clinical and nonhuman isolates. Group 2 strains (n = 6), which included ATCC 8071, were chiefly distinguished from group 1 strains by the inability to oxidize sucrose and maltose. Again, half of these strains originated from clinical material. Group 3 strains (n = 9), all of environmental origin (Lake Michigan area), differed dramatically from groups 1 and 2. They grew poorly or failed to grow at 35°C, produced chitinase, and were nonpigmented on

TABLE 4. Separation of *S. alga* and *S. putrefaciens* by fatty acid analysis

Fatty acid		% (Range) total fatty acid content	
Name	Formula	*S. alga* (n = 7)	*S. putrefaciens* (n = 7)
Pentadecanoic	15:0	26 (22–35)	18 (14–23)
Hexadecanoic	16:0	7 (6–9)	11 (9–13)
None	17:1ω8c	15 (10–17)	12 (9–15)
Dodecanoic	12:0	2 (1–2)	3 (2–4)

TABLE 5. Biogroups of *S. putrefaciens*

Group	Characteristic (% positive)[a]							
	Growth at 35°C	α-Glucosidase activity	Chitinase activity	Pigmentation on L-tryptophan	Utilization of urocanic acid	Acid produced from:		
						Maltose	Arabinose	Sucrose
1	+ (100)	− (0)	− (13)	+ (88)	+ (100)	+ (100)	+ (100)	+ (100)
2	+ (100)	− (0)	− (0)	+ (83)	V (67)	− (17)	+ (83)	− (0)
3	− (11)	V (27)	+ (100)	− (0)	− (0)	+ (100)	− (0)	− (0)

[a] V, variable.

L-tryptophan agar. All nine group 3 strains initially produced α-glucosidase, but upon retesting only three strains were consistently positive. Maltose was oxidized, but not sucrose or arabinose. Unlike groups 1 and 2, urocanic acid was not utilized as a source of energy.

Recently, Vogel and colleagues (21) have noted differences between *S. alga* and *S. putrefaciens* in their susceptibilities to certain antimicrobial agents, including penicillin, ampicillin, and tetracycline. This, coupled with the report linking hemolytic activity with *S. alga* and its apparent association with human disease, suggests possible differences in pathogenicity between these two species (Table 6). We therefore selected 10 strains (5 from each species) for further analysis. Although we did not notice major differences in susceptibility to penicillin, ampicillin, and tetracycline between these two groups based upon the susceptibility category (susceptible, intermediate, or resistant), we did notice that the mean MICs for *S. alga* of penicillin, ampicillin, and tetracycline (~200, 56, and 5.2 μg/ml, respectively) were greater than the corresponding MICs for *S. putrefaciens* (3, 1.3, and 1.1 μg/ml, respectively).

Four of five *S. putrefaciens* strains attached weakly (+) to strongly (+++) (Table 6) to HEp-2 cells in adherence assays; in contrast, no *S. alga* strains exhibited similar adhesive characteristics, although four strains bound strongly to the glass slide background. Invasive activities were not detected in any *Shewanella* strain. Although a delayed hemolytic reaction was observed on sheep blood agar for all five *S. alga* strains (Table 2), beta-hemolysis was not detected with the agar overlay technique or broth assays (Table 6); control *E. tarda* strains were positive in 1 h in both tests. For all five *S. alga* strains (and one *S. putrefaciens* isolate), a weak cytotoxic reaction was sometimes observed during adhesion and invasion studies. This cytotoxic reaction was manifested by the appearance of HEp-2 cells with abnormal cellular morphology, including cell debris (ghosts). Differences in mouse pathogenicity between these two species were found, however, as the mean LD_{50} in Swiss Webster mice for *S. alga* was 1.9×10^8 CFU, while that for *S. putrefaciens* was 8.4×10^8 CFU ($P < 0.02$).

DISCUSSION

In agreement with previous investigators (15, 21), we found *S. alga*, rather than *S. putrefaciens*, to be the predominant *Shewanella* species associated with clinical specimens. These species were easy to distinguish from one another by using a number of recommended phenotypic tests, such as growth at 42°C, growth in 6.5% NaCl, production of a hemolytic substance, and utilization of various carbohydrates (Table 2). Growth on SS agar, a differential trait noted in various studies, was found to be less useful in the present survey, as 52% of the *S. putrefaciens* strains exhibited this characteristic. However, this result may be a somewhat spurious observation, as most SS-positive *S. putrefaciens* strains originated from one geographic region (Lake Michigan and Green Bay). Although the *S. alga* strains typically were unable to utilize sugars, 35% of the isolates tested produced acid from D-ribose. Domínguez and colleagues (5) also found that two bacteremic strains of *S. alga*, plus the type strain, used D-ribose, while the type strain of *S. putrefaciens* did not. Thus, production of acid from D-ribose may be a marker for *S. alga* strains. Another species-associated trait was alkylsulfatase activity; >70% of the *S. putrefaciens* strains elaborated this enzyme, while only 8% of the *S. alga* strains were positive. We also found the *S. alga* strains to produce uniformly higher levels of enzymatic activities

TABLE 6. Virulence properties of *Shewanella* species

Species and strain	MIC (category)[a]			Hemolysis		HEp-2 cell characteristic[c]			LD_{50} (CFU)
	Penicillin	Ampicillin	Tetracycline	Plate	Tube/AO[b]	Adherence	Cytotoxicity	Invasion	
S. alga									
SP-1	>256 (R)	4 (S)	4 (S)	+	−	−	(−)	−	1.2×10^8
SP-2	>256 (R)	>256 (R)	16 (R)	+	−	−	(−)	−	2.5×10^8
SP-4	>256 (R)	3 (S)	2 (S)	+	−	−	(−)	−	2.6×10^8
SP-5	>256 (R)	16 (I)	2 (S)	+	−	−	(−)	−	3.0×10^8
SP-6	3 (R)	0.5 (S)	2 (S)	+	−	−	(−)	−	1.7×10^7
S. putrefaciens									
SP-3	2 (S)	1 (S)	1 (S)	−	−	−	(−)	−	7.1×10^8
SP-7	3 (R)	1.5 (S)	0.75 (S)	−	−	+++	−	−	1.3×10^9
SP-22	3 (R)	2 (S)	1.5 (S)	+	−	+++	−	−	5×10^8
SP-31	4 (R)	1 (S)	1.5 (S)	−	−	++	−	−	6.7×10^8
SP-32	3 (R)	1 (S)	1 (S)	−	−	+	−	−	1×10^9

[a] Values are in micrograms per milliliter. R, resistant; S, susceptible; I, intermediate.
[b] AO, agar overlay.
[c] The number of plus signs indicates the relative degree of adherence. Parentheses indicate HEp-2 cells that showed minimal evidence of a cytotoxic reaction.

against a number of substrates in the API-ZYM system (Table 3). Of nine compounds utilized by either or both species, *S. alga* produced approximately two- to threefold-higher mean enzymatic activity against five of these substrates than did *S. putrefaciens*. This suggests that isolates might be presumptively screened for species determinations with this 4-h test, based upon the overall higher enzymatic activity of *S. alga* strains. Finally, we also noted that comparisons of fatty acid profiles of the two species revealed quantitative differences in four fatty acids that may be useful in species determination (Table 4). However, both of the latter sets of results need confirmation by evaluation of a larger number of isolates of each species.

A reflection of the phenotypic diversity found within *S. putrefaciens* was the recognition of three distinct biogroups within the species (Table 5). These biogroups were distinguished from one another by a number of phenotypic tests, including several new differential characteristics (α-glucosidase, chitinase, and urocanic acid). Nozue and others (15) found that *S. putrefaciens* as presently defined is still heterogeneous at the DNA level, with at least three detectable genomospecies based upon DNA homology values. Clearly, further work needs to be undertaken to link specific phenotypic markers to each of these hybridization groups.

Why *S. alga* appears to be the predominant *Shewanella* species associated with human infections remains unclear. Results of limited pathogenicity studies on select *Shewanella* strains suggest that adherence to epithelial cells is not a major determinant. However, we did notice significant differences between *S. alga* and *S. putrefaciens* in mouse LD_{50} studies, suggesting a possible explanation for the preferential association of the former species with human disease. One attractive explanation for this observation is the hemolytic reaction produced by virtually all *S. alga* strains, and some authors have suggested possible exotoxin involvement in *S. putrefaciens* cellulitis (3). Although we were able to detect this beta-hemolytic-type reaction upon prolonged incubation of isolates on sheep blood agar (48 to 72 h), other methods traditionally positive for the presence of a cytolytic toxin (beta-hemolysin), such as broth, agar overlay, and epithelial cell cytotoxicity assays, were either uniformly negative or weakly positive at best. Such results raise considerable questions as to the nature of this hemolytic reaction and whether or not it is caused by a true beta-hemolysin. Regardless, there appears to be increasing evidence that, between *S. alga* and *S. putrefaciens*, *S. alga* causes the most human illnesses and that significant differences exist between these two species regarding resistance to antimicrobial agents, mouse pathogenicity, and certain virulence factors (hemolysis and adhesion). Whether these factors translate into true in vivo differences with respect to disease spectrum, pathogenicity, and therapy remains to be determined.

REFERENCES

1. **Brink, A. J., A. van Straten, and A. J. van Rensburg.** 1995. *Shewanella* (*Pseudomonas*) *putrefaciens* bacteremia. Clin. Infect. Dis. **20:**1327–1332.
2. **Butt, A. A., J. Figueroa, and D. H. Martin.** 1997. Ocular infection caused by three unusual marine organisms. Clin. Infect. Dis. **24:**470.
3. **Chen, S. C. A., R. H. Lawrence, D. R. Packlam, and T. C. Sorrell.** 1991. Cellulitis due to *Pseudomonas putrefaciens*: possible production of exotoxins. Rev. Infect. Dis. **13:**642–643.
4. **Chen, Y.-S., Y.-C. Liu, M.-Y. Yen, J.-H. Wang, J.-H. Wang, S.-R. Wann, and D.-L. Cheng.** 1997. Skin and soft-tissue manifestations of *Shewanella putrefaciens* infection. Clin. Infect. Dis. **25:**225–229.
5. **Domínguez, H., B. F. Vogel, L. Gram, S. Hoffmann, and S. Schaebel.** 1996. *Shewanella alga* bacteremia in two patients with lower leg ulcers. Clin. Infect. Dis. **22:**1036–1039.
6. **Gilardi, G. L.** 1985. Cultural and biochemical aspects for identification of glucose-nonfermenting gram-negative rods, p. 17–84. *In* G. L. Gilardi (ed.), Nonfermentative gram-negative rods. Marcel Dekker, Inc., New York, N.Y.
7. **Graves, M., T. Robin, A. M. Chipman, J. Wong, S. Khashe, and J. M. Janda.** 1997. Four additional cases of *Burkholderia gladioli* infection with microbiological correlates and review. Clin. Infect. Dis. **25:**838–842.
8. **Janda, J. M.** 1985. Biochemical and exoenzymatic properties of *Aeromonas* species. Diagn. Microbiol. Infect. Dis. **3:**223–232.
9. **Janda, J. M., and S. L. Abbott.** 1993. Expression of an iron-regulated hemolysin by *Edwardsiella tarda*. FEMS Microbiol. Lett. **111:**275–280.
10. **Janda, J. M., S. L. Abbott, S. Khashe, G. H. Kellogg, and T. Shimada.** 1996. Further studies on biochemical characteristics and serologic properties of the genus *Aeromonas*. J. Clin. Microbiol. **34:**1930–1933.
11. **Janda, J. M., S. L. Abbott, S. Khashe, and T. Robin.** 1996. Biochemical investigations of biogroups and subspecies of *Morganella morganii*. J. Clin. Microbiol. **34:**108–113.
12. **Janda, J. M., and R. P. Kokka.** 1991. The pathogenicity of *Aeromonas* strains relative to genospecies and phenospecies identification. FEMS Microbiol. Lett. **90:**29–34.
13. **Kim, J. H., R. A. Cooper, K. E. Welty-Wolf, L. J. Harrell, P. Zwadyk, and M. E. Klotman.** 1989. *Pseudomonas putrefaciens* bacteremia. Rev. Infect. Dis. **11:**97–104.
14. **Levin, R. E.** 1972. Correlation of DNA base composition and metabolism of *Pseudomonas putrefaciens* isolates from food, human clinical specimens, and other sources. Antonie Leeuwenhoek **38:**121–127.
15. **Nozue, H., T. Hayashi, Y. Hashimoto, T. Ezaki, K. Hamasaki, K. Ohwada, and Y. Terawaki.** 1992. Isolation and characterization of *Shewanella alga* from human clinical specimens and emendation of the description of *S. alga* Simidu et al., 1990, 335. Int. J. Syst. Bacteriol. **42:**628–634.
16. **Oliver, J. D., J. E. Wear, M. B. Thomas, M. Warner, and K. Linder.** 1986. Production of extracellular enzymes and cytotoxicity by *Vibrio vulnificus*. Diagn. Microbiol. Infect. Dis. **5:**99–111.
17. **Owen, R. J., R. M. Legros, and S. P. Lapage.** 1978. Base composition, size and sequence similarities of genome deoxyribonucleic acids from clinical isolates of *Pseudomonas putrefaciens*. J. Gen. Microbiol. **104:**127–138.
18. **Semple, K. M., J. L. Doran, and D. S. Westlake.** 1989. DNA relatedness of oil-field isolates of *Shewanella putrefaciens*. Can. J. Microbiol. **35:**925–931.
19. **Simidu, U., K. Kita-Tsukamoto, T. Yasumoto, and M. Yotsu.** 1990. Taxonomy of four marine bacterial strains that produce tetrodotoxin. Int. J. Syst. Bacteriol. **40:**331–336.
20. **Stenström, I.-M., and G. Molin.** 1990. Classification of the spoilage flora of fish, with special reference to *Shewanella putrefaciens*. J. Appl. Bacteriol. **68:**601–618.
21. **Vogel, B. F., K. Jørgensen, H. Christensen, J. E. Olsen, and L. Gram.** 1997. Differentiation of *Shewanella putrefaciens* and *Shewanella alga* on the basis of whole-cell protein profiles, ribotyping, phenotypic characterization, and 16S rRNA gene sequence analysis. Appl. Environ. Microbiol. **63:**2189–2199.
22. **Weyant, R. S., C. W. Moss, R. E. Weaver, D. G. Hollis, J. G. Jordan, E. C. Cook, and M. I. Daneshvr.** 1995. Identification of unusual pathogenic gram-negative aerobic and facultatively anaerobic bacteria, 2nd ed. Williams & Wilkins, Baltimore, Md.